

AMENDMENTS TO THE SPECIFICATION

On page 10, please replace the paragraph beginning on line 17 and ending on line 18 with the following amended paragraph:

Figure 5. Human Telomerase Reverse Transcriptase (hTRT) sequence (SEQ ID NO:23) [from Nakamura *et al.*, 1997].

On page 10, line 24, insert the following new paragraph:

Figure 7 shows an exemplary diagram of the interaction between a cancer cell, T-cell, and killer cells.

On page 14, please replace the paragraph beginning on line 4 and ending on line 10 with the following amended paragraph:

hTRT synthetic peptides p540 (540ILAKFLHWL548) (SEQ ID NO:1), p865 (865RLVDDFLLV873) (SEQ ID NO:2) and MART-1 (27AAGIGILTV35) (SEQ ID NO:3) were purchased from the Biopolymer Synthesis Center (CalTech, Pasadena, CA). Synthetic peptides 128TPPAYRPPNAPIL140 (SEQ ID NO:4) of the hepatitis B core antigen (HBVc), 571YLSGANLNL579 (SEQ ID NO:5) of carcinoembryonic antigen (CEA), 476VLRYGVSFSV486 (SEQ ID NO:6) of melanoma antigen gp 100, 476ILKEPVHGV484 (SEQ ID NO:7) of HIV- I reverse transcriptase were purchased from Neosystem (Strasburg, France).

On page 16, please replace the paragraph beginning on line 1 and ending on line 5 with the following amended paragraph:

HHD mice were immunized subcutaneously at the base of the tail with 100 µg of individual hTRT peptide emulsified in incomplete Freund's adjuvant (IFA). Half of the mice were immunized with the hTRT peptide and 140 µg of the helper peptide TPPAYRPPNAPIL (SEQ ID NO:4), which corresponds to residues 128-140 of the hepatitis B core antigen (HBVc) (25).

On page 16, please replace the paragraph beginning on line 8 and ending on page 17, line 6 with the following amended paragraph:

The relative avidity was measured as previously described (25). Briefly, T2 cells were incubated overnight at 37°C in RPMI supplemented with human β 2-microglobulin (100 ng/ml) (Sigma, St. Louis, MO) in the absence (negative control) or presence of the test peptide or the reference peptide 476ILKEPVHGV484 (SEQ ID NO:7) of HIV-1 reverse transcriptase at various final peptide concentrations (0.1-100 μ M). Cells were incubated with Brefeldin A (0.5 μ g/ml) for one hour and subsequently stained with a saturating concentration of monoclonal antibody BB7.2 for 30 minutes at ~~+4°C~~ +4°C followed by washing and a second incubation with a goat antibody to mouse Ig (Fab')₂ conjugated to FITC (Caltag, South San Francisco). Cells were then washed, fixed with 1% paraformaldehyde and analyzed in a FACs Calibur cytofluorimeter (Becton&Dickinson, San Jose, CA). The mean fluorescence intensity of each concentration minus that of cells without peptide was used as an estimate of peptide binding. Results are expressed as values of RA, which is the ratio of the concentration of test peptide necessary to reach 20% of the maximal binding by the reference peptide over that of the reference peptide so that the lower the value the stronger the binding. Dissociation of the test peptide from the HLA-A2.1 molecule reflects the half-life of fluorescence intensity of the peptide/MHC complex over time. The half-life of the complex (DC50) refers to the time (hours) required for a 50% reduction of the T0 mean fluorescence intensity (25). Synthetic peptides 571YLSGANLNL579 (SEQ ID NO:5) of carcinoembryonic antigen (CEA) and 476VLYRYGSFSV486 (SEQ ID NO:6) of melanoma antigen gp100 were used as internal controls to account for inter-tests variability and for consistency with previously reported RA and DC50 measures (25).

On page 18, please replace the paragraphs beginning on line 4 and ending on page 20, line 3 with the following amended paragraphs:

The amino acid sequence of hTRT (locus AF015950) (19) was analyzed for 9mer peptide sequences containing known binding motifs for the HLA-A2.1 molecule [52; 35; 60], a subtype encompassing 95% of HLA-A2 allele which is expressed in about 50%

of the Caucasian population (26-28). Peptides were identified by reverse genetics based on canonical anchor residues for HLA-A2.1 (29), and by using the software of the Bioinformatics & Molecular Analysis Section (NIH) available at http://bimas.dcrt.nih.gov/molbio/hla_bind/index.html which ranks 9mer peptides on a predicted half-time dissociation coefficient from HLA Class I molecules (30). From an initial panel of ~30 candidate peptides Applicant retained two sequences, 5401LAKFLHWL548 (SEQ ID NO:1) and 865RLVDDFLLV873 (SEQ ID NO:2), denoted hereunder as p540 and p865.

Since the immunogenicity of MHC Class I-restricted peptides reflects to some degree their binding and stabilizing capacity for MHC Class I molecules (31-33) Applicant sought direct proof of the strength of interaction between the two hTRT peptides and the HLA-A2.1 molecule in a conventional binding/stabilization assay that uses the antigen-transporting deficient (TAP-) HLA-A2.1+ human T2 cells. The relative avidity (RA) calculated in reference to 476ILKEPVHGV484 (SEQ ID NO:7) of HIV-1 reverse transcriptase, a canonical high binder peptide (25), was 2.9 and 2.5 for p540 and p865, respectively (Table 1). The stability of each peptide bound to HLA-A2.1 was measured as the half-life of the complex (DC50) and was in the order of 4-6 hours for

TABLE 1

Induction of CTL Against hTRT in HLA-A2.1 Transgenic Mice

Group	hTRT Peptide	Helper Peptide	No. Responders	Percent lysis
I	5401LAKFLHWL548 (<u>SEQ ID NO:1</u>)	-	10/15 (66%)	(35,21,34,42,56,21,12,35,42,16)
II	"	+	8/10 (80%)	(45,56,62,64,65,45,65,45)
III	865RLVDDFLLV873 (<u>SEQ ID NO:2</u>)	-	3/15 (20%)	(25,12,15)
IV	"	+	7/10 (70%)	(25,32,35,12,16,18,21)

- a. HHD mice were immunized by a subcutaneous injection of 100 µg of hTRT peptide emulsified in incomplete Freund's adjuvant (IFA). In group 2 and 4 the hTRT peptide was administered together with 140 µg of the helper peptide TPPAYRPPNAPIL (SEQ ID NO:4) (25).

- b. Values of cytotoxicity refer to individual responder mice. Spleen-derived CTL were harvested 7 days after immunization and then cultured for six days with the homologous hTRT peptide. Values refer to maximal cytotoxicity at an effector to target ratio of 60:1.

p540 and 2-4 hours for p865, respectively. Collectively, these measurements indicate that both hTRT peptides are excellent binders to HLA-A2.1 albeit p865 has a faster dissociation rate.

On page 23, please replace the paragraph beginning and ending on line 8 with the following amended paragraph:

Example 44 9.

On page 23, please replace the paragraph beginning on line 25 and ending on page 25, line 5 with the following amended paragraph:

The antigen-recognition activity of T cells is intimately linked with

Table III
hTRT-Derived HLA-A2.1-Restricted Peptides

Anchor Position L at position 2 V at position 9	Anchor Position L at position 2 V at position 9	Anchor Position M at position 2 V, L or I at position 9
¹⁵² LLARCALFV ¹⁶⁰ (SEQ ID NO:8)	⁹⁶ VLAFGFALL ¹⁰⁴ (SEQ ID NO:9)	⁸¹²FMCHHAVRI⁸²⁰ ⁸¹² FMCHHAVRI ⁸²⁰ (SEQ ID NO:17)
⁸⁶⁵ RLVDDFLLV ⁸⁷³ (SEQ ID NO:2)	⁶⁷⁵ LLGASVLGL ⁶⁸³ (SEQ ID NO:10)	
	⁷²⁴ RLTEVIASI ⁷³² (SEQ ID NO:11)	
	⁷⁹⁷ SLNEASSGL ⁸⁰⁵ (SEQ ID NO:12)	
	⁸³⁶ ILSTLLCSL ⁸⁴¹ (SEQ ID NO:13)	
	⁹²⁶ GLFPWCGLL ⁹³⁴ (SEQ ID NO:14)	
	¹⁰⁷² WLCHQAFL ¹⁰⁸⁰ (SEQ ID NO:15)	
	⁵⁷² RLFFYRKSY ⁵⁸⁰ (SEQ ID NO:16)	

recognition of MHC (HLA in humans) molecules. This complex is located on chromosome 6, and encompasses nearly 200 genes encoding for MHC class I and class II among others. The

initial discovery is in relation to the HLA-A2 allele which is expressed in about 50% of the Caucasian population (56). About 95% of HLA-A2+ white individuals express the HLA-A2.1 subtype (53).

On page 26, please replace the text beginning with Table IV and ending on line 21 with the following amended text:

Table IV
hTRT-Derived HLA-A2.1-Restricted Peptides

Anchor Position L at position 2 V at position 9	Anchor Position L at position 2 V at position 9	Anchor Position M at position 2 V, L or I at position 9
¹⁵² LLARCALFV ¹⁶⁰ (SEQ ID NO:8) ⁸⁶⁵ RLVDDFLLV ⁸⁷³ (SEQ ID NO:2)	⁹⁶ VLAFGFALL ¹⁰⁴ (SEQ ID NO:9) ⁶⁷⁵ LLGASVLGL ⁶⁸³ (SEQ ID NO:10) ⁷²⁴ RLTEVIASI ⁷³² (SEQ ID NO:11) ⁷⁹⁷ SLNEASSGL ⁸⁰⁵ (SEQ ID NO:12) ⁸³⁶ ILSTLLCSL ⁸⁴¹ (SEQ ID NO:13) ⁹²⁶ GLFPWCGLL ⁹³⁴ (SEQ ID NO:14) ¹⁰⁷² WLCHQAFL ¹⁰⁸⁰ (SEQ ID NO:15)	⁸¹² FMCHHAVRI ⁸²⁰ (SEQ ID NO:17)

Applicant used two such peptides ~~540ILAKFLHWL549~~ 540ILAKFLHWL548 (SEQ ID NO:1) and ⁸⁶⁵RLVDDFLLV⁸⁷³ (SEQ ID NO:2), denoted as p540 and p865. Both peptides are able to induce a CTL response *in vitro* in normal blood donors and in patients with prostate cancer. Applicant has demonstrated that the same peptides are also able to induce a CTL response *in vitro* in patients with melanoma. A synopsis of these studies is shown in Table V.

On page 27, please replace the paragraph beginning with line 26 and ending on page 28, line 15 with the following amended paragraph:

Applicant then proceeded at a single residue (Y) modification in position 1, which is supposed to increase the binding affinity to HLA-A2 and also its immunogenicity (60). The

Table VI

Additional Sequence of Wild Type and Modified hTRT Peptides

Name of Peptide	Wild Type Sequence	Modified Sequence
p152	¹⁵² LLARCALFV ¹⁶⁰ (SEQ ID NO:8)	¹⁵² YLARCALFV ¹⁶⁰ (SEQ ID NO:18)
p555	⁵⁵⁵ ELLRSFFYV ⁵⁶³ (SEQ ID NO:19)	⁵⁵⁵ YELLRSFFYV ⁵⁶³ (SEQ ID NO:20)
p572	⁷²RLFFYRKSV⁵⁸⁰ ⁵⁷²RLFFYRKSV⁵⁸⁰ (SEQ ID NO:21)	⁵⁷² YLFFYRKSV ⁵⁸⁰ (SEQ ID NO:22)

new modified sequences are shown in Table VI.